Detection of Novel Poxvirus from Gray Seal (*Halichoerus grypus*), Germany

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We detected a novel poxvirus from a gray seal (*Halichoerus grypus*) from the North Sea, Germany. The juvenile animal showed pox-like lesions and deteriorating overall health condition and was finally euthanized. Histology, electron microscopy, sequencing, and PCR confirmed a previously undescribed poxvirus of the *Chordopoxvirinae* subfamily, tentatively named Wadden Sea poxvirus.

Members of the poxvirus subfamily *Chordopoxvirinae* (family *Poxviridae*) infect vertebrates, such as birds, reptiles, and a broad spectrum of mammals. Although some chordopoxviruses have a narrow host range, several can easily jump species barriers and cause severe disease (*1*). Considering the potential zoonotic and epizootic potential of chordopoxviruses, constant monitoring and adaptation of diagnostic procedures are essential. With the advent of metagenomic sequencing, novel chordopoxviruses have been identified that are genetically diverse and were not readily detectable by using established PCR-based diagnostics (*2*–*4*).

We report a case of a poxvirus infection in a gray seal (*Halichoerus grypus*) from the North Sea near Germany. We identified a novel chordopoxvirus that was phylogenetically divergent from other known poxviruses of gray seals.

Case Study

In June 2020, a juvenile gray seal was nursed at a rehabilitation center in Friedrichskoog, Germany (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/6/22-1817-App1.pdf) and was about to be released back into the wild when staff noticed pox-like lesions on its hind flipper (Appendix Figure 2). The seal's overall health condition deteriorated over the next 3 weeks, and it had dyspnea, vomiting after feeding, and apathy; it was humanely euthanized. At necropsy, the seal was in good body condition (abdominal blubber ≈35 mm, reference >30 mm). We noted 2 prominent verrucous nodules on the right hind flipper (Appendix Figure 2). We also found severe emphysema of the mediastinum and a focal, adhesive pleuritis. No other organs had lesions. Histologic examination of both nodules (Appendix) revealed severe papillary epithelial hyperplasia, acanthosis, ballooning degeneration, large eosinophilic cytoplasmatic inclusion bodies in the stratum spinosum, moderate hyperkeratosis, and severe ulceration with hemorrhages (Figure 1, panels A, B). In addition, we observed multifocal severe infiltrations of neutrophils. In the liver, we detected a focally necrotizing hepatitis with ballooning degeneration of nuclei and a focal granulomatous subcapsular hepatitis with intralesional parasites and calcification. We found further inflammatory changes in the lungs (Figure 1, panel C), which had multifocal moderate pneumonia with infiltration of mononuclear cells and neutrophils; the heart had focal severe mononuclear myocarditis; and the duodenum had moderate diffuse lymphoplasmacellular enteritis. We also observed depletion of lymphocytic organs, including a severe atrophy of the thymus.

Results of quantitative PCRs (qPCRs) specific for orthopoxvirus (*5*) and parapoxvirus (*6*), canine alphaherpesvirus 1, influenza A virus, canine morbillivirus, and *Brucella* sp. were negative for lung and skin lesion tissue. We isolated an *Escherichia coli* strain from lung, mediastinum, liver, kidney, and intestines.

We used electron microscopy to analyze lung tissue and detected typical poxvirus-like virions, which were ovoid in shape and ≈250 nm long and ≈200 nm wide (Figure 1, panel D). The surface structures resembled typical orthopox-like randomly arranged tubular

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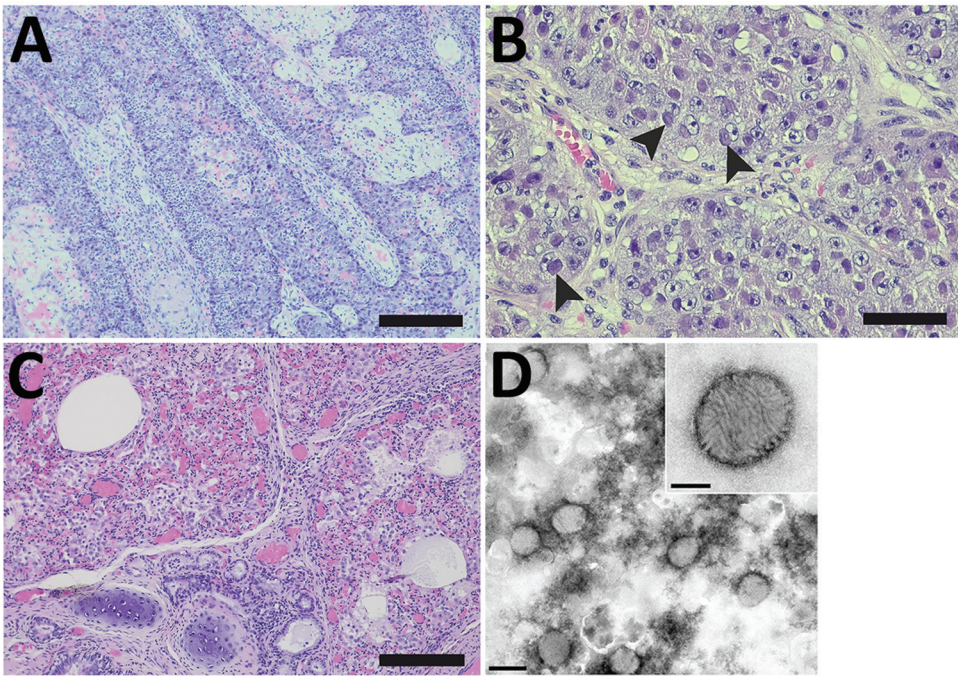

Figure 1. Histopathology and electron microscopy of nodules and lung tissue from a gray seal (*Halichoerus grypus*) with novel poxvirus, North Sea, Germany. A) Histopathology of nodules shows severe papillary epithelial hyperplasia with infiltration of neutrophils. Scale bar indicates 200 µm. B) Histopathology of ballooning degeneration of epithelial cells. Arrows indicate large eosinophilic intracytoplasmic inclusion bodies. Scale bar indicates 50 µm. C) Histopathology of the lung shows multifocal moderate pneumonia with infiltration of mononuclear cells and neutrophils with proliferation of pneumocytes type II and intra-alveolar histiocytosis, severe atelectasis, and hyperemia. Scale bar indicates 100 µm. D) Negative-contrast electron microscopy of lung tissue. Microscopy revealed poxvirus-like viral particles. Scale bar indicates 300 nm. Inset: closeup of poxvirus-like particles, which had an oval shape ≈250 nm × ≈200 nm and an irregular surface with randomly arranged tubular structures; scale bar indicates 100 nm.

units. However, the virion morphology did not enable assignment to a poxvirus genus.

We isolated DNA from a pool of lung and skin lesion tissue and sequenced DNA using Ion Torrent S5XL (Thermo Fisher Scientific, https://www.thermofisher.com) (*7*), NovaSeq (Illumina, https://www.illumina.com), and MinION Mk1C (Oxford Nanopore Technologies, https://nanoporetech.com) sequencing technologies (Appendix). We combined the reads in a hybrid assembly, which resulted in a complete poxviral genome (mean coverage ≈650). We were able to confirm completeness of the genome because the terminal repeats contained the terminal hairpin region.

We screened several organs by using 2 different virus-specific qPCRs (Appendix). Results from both qPCR panels were consistent and we detected the highest viral loads in the skin lesion and parts of the lung (Table).

We tentatively named the poxvirus Wadden Sea poxvirus (WSPV) to reflect the geographic origin of the infected gray seal, which was found in the Wadden Sea, an intertidal zone in the southeastern part of the North Sea, Germany (Appendix Figure 1). We submitted the annotated WSPV genome sequence to the International Nucleotide Sequence Database Collaboration (https://www.insdc.org; accession no. OP810554).

WSPV had one of the smallest genomes (124,614 bp) and lowest guanine-cytosine content (≈22.5%) described so far among chordopoxviruses. The unique core genome of 117,842 bp was flanked by 2 inverted terminal repeats of 3,386 bp each. We identified 124 unique potential open reading frames (ORFs), of which 3 were duplicated in the inverted terminal repeats. BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) identified 111 ORFs representing orthologs of poxvirus proteins. Nine ORFs encoded proteins that were not related to known poxvirus proteins but showed sequence similarity to eukaryote proteins, and 4 ORFs remained unclassified.

Table. Quantitative PCR detection of novel poxvirus from different tissues of a gray seal (*Halichoerus grypus*), Germany*

Sample	Cycle quantification value	
	Panel 1, viral DNA polymerase	Panel 2, viral RNA polymerase
Skin lesion	9.1	8.9
Lung 1	18.2	18.2
Lung 2	32.3	31.3
Lymph nodes	25.0	24.6
Uterus	26.5	26.2
Spleen	27.2	27.2
Kidney	28.8	28.8
Blood, EDTA	32.2	31.5
Brain	32.9	33.7
Liver	33.7	32.7

*Novel virus is tentatively named Wadden Sea virus.

For phylogenetic classification, we compared the amino acid sequences encoded by 15 poxvirus core genes from WSPV with the respective homologs from 47 representative poxviruses (Appendix). WSPV formed a separate phylogenetic branch that did not fall within any of the established genera (Figure 2) and likely is a new species within a novel genus of the subfamily *Chordopoxvirinae*. Of note, a sequence comparison of the predicted WSPV DNA polymerase protein to the nonredundant BLAST database revealed a 96.3% sequence identity with a partial sequence from a Steller sea lion poxvirus (GenBank accession no. AAR06586.1), but other poxviruses had a sequence identity <77%.

Conclusions

Infections with poxviruses have been reported from gray seals and harbor seals (*Phoca vitulina*), both of which live in the North Sea, Germany. Poxviruses have also been reported in other pinniped species and infections are usually associated to subclades of parapoxviruses, called sealpox or sea lion pox virus (*8*–*10*). Ulcerative to proliferative, nodular, cutaneous, and mucosal lesions have been found in seals infected with parapoxviruses (*10*–*12*). The nodules usually heal spontaneously, but healing lasts from several weeks up to a few months. The illness rate is high, but death rates are low (*13*). Rarely, nodules in

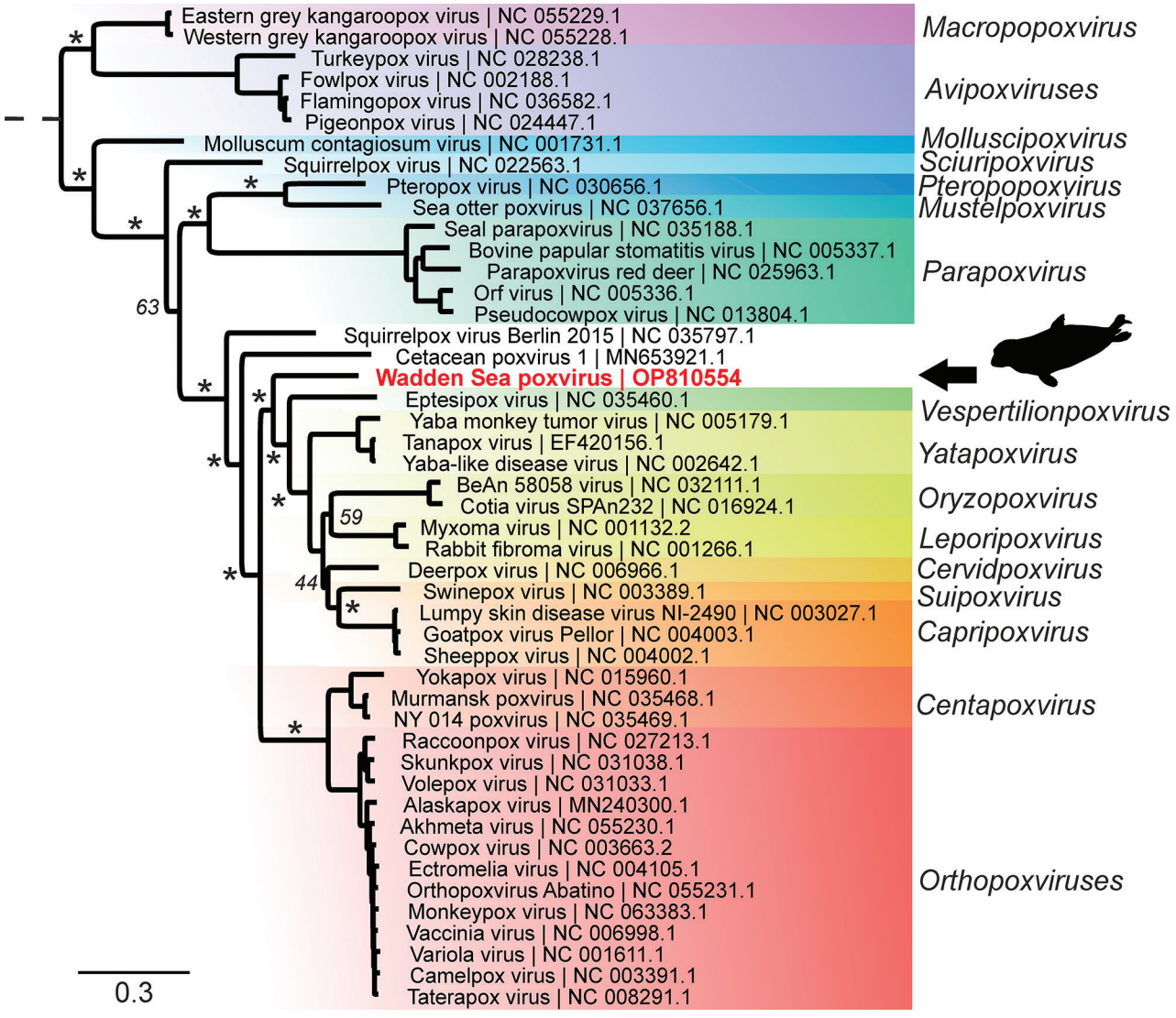

Figure 2. Phylogenetic tree of novel poxvirus detected from gray seal (*Halichoerus grypus*), Germany. Sequencing resulted in a complete poxvirus genome and the virus was tentatively named Wadden Sea poxvirus (red text). Phylogenetic analysis of 15 concatenated viral proteins (alignment of 9,130 aa) showed that Wadden Sea poxvirus (black arrow) is a member of the subfamily *Chordopoxvirinae* but might resemble a novel species distant from the established genera. Asterisks indicate major branches of the bootstrap support at >90%. Scale bar indicates amino acid substitutions per site.

the oral cavity can lead to problems during feeding, and secondary bacterial infection can fatally impair respiratory functions (*14*). However, in the case we report, we could not link the animal's overall deteriorating health condition and severe pneumonia to oral lesions. We considered the cultured *E. coli* strain a facultative pathogen that might have been involved in disease; however, postmortem contamination might be more likely.

We did not detect any parapoxvirus DNA, but sequencing revealed WSPV, a novel poxvirus that is phylogenetically distinct from any other members of the subfamily *Chordopoxvirinae*. The high loads of viral DNA in several organs (Table), and the observed histopathologic changes suggested a generalized infection with systemic pathology. Lesion associated detection of high viral loads (cycle quantification [cq] values for skin cq ≈9, for lung cq ≈18) indicated that the WSPV infection was likely responsible for the gray seal's disease and severe pneumonia. However, other factors might have been involved, and the source of infection, the potential natural reservoir, and the zoonotic potential of WSPV are unknown. None of the contact animals within the rehabilitation center had similar lesions develop and so far, no further cases have been reported.

Sequence comparison of WSPV showed that a close relative of this novel poxvirus has been detected in a cutaneous lesion of a young Steller sea lion (*Eumetopias jubatus*) from Prince William Sound, Alaska, USA (*15*). This finding suggests a geographically wide distribution of WSPV or WSPV-related viruses and the potential to infect other pinnipeds. As noted in the case we describe, WSPV can cause severe disease. Therefore, future diagnostic considerations for pox-like lesions of pinniped species should include WSPV.

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References

1. McFadden G. Poxvirus tropism. Nat Rev Microbiol. 2005;3:201–13. https://doi.org/10.1038/nrmicro1099
2. Hodo CL, Mauldin MR, Light JE, Wilkins K, Tang S, Nakazawa Y, et al. Novel poxvirus in proliferative lesions of wild rodents in east central Texas, USA. Emerg Infect Dis. 2018;24:1069–72. https://doi.org/10.3201/eid2406.172057
3. David D, Davidson I, Berkowitz A, Karniely S, Edery N, Bumbarov V, et al. A novel poxvirus isolated from an Egyptian fruit bat in Israel. Vet Med Sci. 2020;6:587–90. https://doi.org/10.1002/vms3.233
4. Wibbelt G, Tausch SH, Dabrowski PW, Kershaw O, Nitsche A, Schrick L. Berlin squirrelpox virus, a new poxvirus in red squirrels, Berlin, Germany. Emerg Infect Dis. 2017;23:1726–9. https://doi.org/10.3201/eid2310.171008
5. Olson VA, Laue T, Laker MT, Babkin IV, Drosten C, Shchelkunov SN, et al. Real-time PCR system for detection of orthopoxviruses and simultaneous identification of smallpox virus. J Clin Microbiol. 2004;42:1940–6. https://doi.org/10.1128/JCM.42.5.1940-1946.2004
6. Nitsche A, Büttner M, Wilhelm S, Pauli G, Meyer H. Real-time PCR detection of parapoxvirus DNA. Clin Chem. 2006;52:316–9. https://doi.org/10.1373/clinchem.2005.060335
7. Wylezich C, Papa A, Beer M, Höper D. A versatile sample processing workflow for metagenomic pathogen detection. Sci Rep. 2018;8:13108. https://doi.org/10.1038/s41598-018-31496-1
8. Tryland M, Klein J, Nordøy ES, Blix AS. Isolation and partial characterization of a parapoxvirus isolated from a skin lesion of a Weddell seal. Virus Res. 2005;108:83–7. https://doi.org/10.1016/j.virusres.2004.08.005
9. Costa H, Klein J, Breines EM, Nollens HH, Matassa K, Garron M, et al. A comparison of parapoxviruses in North American pinnipeds. Front Vet Sci. 2021;8:653094. https://doi.org/10.3389/fvets.2021.653094
10. Günther T, Haas L, Alawi M, Wohlsein P, Marks J, Grundhoff A, et al. Recovery of the first full-length genome sequence of a parapoxvirus directly from a clinical sample. Sci Rep. 2017;7:3734. https://doi.org/10.1038/s41598-017-03997-y
11. Roess AA, Levine RS, Barth L, Monroe BP, Carroll DS, Damon IK, et al. Sealpox virus in marine mammal rehabilitation facilities, North America, 2007–2009. Emerg Infect Dis. 2011;17:2203–8. https://doi.org/10.3201/eid1712.101945
12. Müller G, Gröters S, Siebert U, Rosenberger T, Driver J, König M, et al. Parapoxvirus infection in harbor seals (*Phoca vitulina*) from the German North Sea. Vet Pathol. 2003;40:445–54. https://doi.org/10.1354/vp.40-4-445
13. Hicks BD, Worthy GA. Sealpox in captive grey seals (*Halichoerus grypus*) and their handlers. J Wildl Dis. 1987;23:1–6. https://doi.org/10.7589/0090-3558-23.1.1
14. Tryland M. Seal parapoxvirus. In: Liu D, editor. Molecular detection of human viral pathogens, 1st edition. New York: CRC Press; 2010. p. 1029–38.
15. Bracht AJ, Brudek RL, Ewing RY, Manire CA, Burek KA, Rosa C, et al. Genetic identification of novel poxviruses of cetaceans and pinnipeds. Arch Virol. 2006;151:423–38. https://doi.org/10.1007/s00705-005-0679-6

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Appendix

Materials and Methods

Histopathology

For histology, tissue samples of skin, lung, heart, thymus, mediastinum, liver, spleen, kidney, urinary bladder, esophagus, stomach, intestine, pancreas, ovary, bone marrow, brain, and lymph nodes were collected and fixed in 10% non-buffered formalin, processed by routine methods, embedded in paraffin wax, sectioned at 2 µm and stained with hematoxylin and eosin (HE) using standard methods.

Nucleic Acid Extraction

Prior to DNA extraction, ≈50 mg of native organ samples were homogenized using the TissueLyser II (Qiagen, Germany) in combination with 1 mL cell culture medium (mixture of equal volumes of Eagle Minimum Essential Medium (MEM) (Hank's balanced salts solution) and Eagle MEM (Earle's balanced salts solution), 2 mM L-Gln, nonessential amino acids, adjusted to 850 mg/L $NaHCO_3$, 120 mg per L sodium pyruvate, pH 7.2 with 10% FCS (Bio & Sell GmbH, Germany) and a stainless-steel bead.

For metagenomic sequencing, a pool of homogenized lung and skin lesion tissue was centrifuged and the pellet was collected in 1 mL LBE buffer. Total DNA then was extracted using the RNAdvance Tissue kit (Beckman Coulter, Germany) in combination with a KingFisher Flex system (Thermo Fisher Scientific, Germany) skipping the DNase I digestion step.

For screening different organs for presence of viral DNA by qPCR, supernatants from the homogenization step were collected. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions.

Electron Microscopy

For transmission electron microscopy, tissue from the lung was homogenized and diluted in media. The suspension was used for adhesion to Formvar coated EM grids (Ni, 400 mesh). The EM grids were additional coated with carbon and glow discharged before adhesion to the sample. 1% phosphor tungstic acid pH 6 was used for negative staining and the samples were analyzed by a Talos F200i transmission electron microscope (Thermo Fisher Scientific) at an acceleration voltage of 80 kV. The used detector is the Ceta 16M Camera together with Velox Imaging Software (Thermo Fisher Scientific).

Sequencing

The DNA from a pool of lung and skin lesion tissue was sequenced using 3 different platforms: Ion Torrent, nanopore, and Illumina.

For Ion Torrent, DNA was used for library preparation as described in Wylezich et al. 2018 (*1*). The library was then sequenced using an Ion Torrent S5XL system (Life Technologies, Germany).

For nanopore long read sequencing, DNA was prepared by using an SQK-RBK004 Rapid Barcoding Kit (Oxford Nanopore Technologies, UK) following the manufactures instructions. The final library was run on a MinION Flow Cell (R9.4.1) for 19h 46m using a Mk1C device (Oxford Nanopore Technologies, UK).

For Illumina, DNA was submitted to Eurofins Genomics Germany and sequenced using a NovaSeq instrument (Illumina, USA) running in paired-end mode (2 × 150 bp).

Sequence Analysis

Raw Illumina paired-end reads were trimmed to remove bad quality regions and adaptor contamination using TrimGalore! version 0.6.5. A subset of the trimmed paired-end reads along with the Nanopore long reads were de novo assembled using SPAdes version 3.15.4; (*2*) and Unicycler version 0.4.4, both running in hybrid mode. The resulting contigs were visualized, arranged, and annotated using Geneious Prime version 2021.0.1. Open reading frames were predicted and translated, and subsequently classified using BLASTp version 2.10.1 against a filtered version of the “nr” database that contained only entries belonging to the taxonomic superkingdoms “Viruses” (NCBI:txid10239) and “Eukaryota” (NCBI:txid2759).

Phylogenetic Analysis

The amino acid (aa) sequences of 15 predicted ORFs from Wadden Sea poxvirus (WSPV) were aligned to their respective homologs (Appendix Table 1) from 47 representative poxviruses. Individual amino acid alignments were generated using MUSCLE version 3.8.425 (*3*). The alignments were subsequently concatenated and used for phylogenetic analysis using IQ-TREE2 version 2.2.0 (*4*). In detail, we used a partition model (*-p*) to reflect the nature of the multi gene alignment allowing for individual substitution models and evolutionary rates (*5*). The optimal substitution model for each partition was selected using ModelFinder, *-m MFP* (*6*). Branch support was calculated using 100,000 ultra-fast bootstraps, *-bb 100000* (*7*).

Quantitative PCR

Two sets of specific primer/probe sets (Appendix Table 2) for real-time quantitative PCR were designed using Primer3web version 4.1.0 targeting the viral DNA- and RNA-polymerase genes of the WSPV genome. The AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, Germany) was used according to the manufacturer's instructions and the reaction was performed with the following cycler setup: 95°C for 10 min, 42 cycles of 95°C for 15 sec, 57°C for 20 sec.

References

Wylezich C, Papa A, Beer M, Höper D. A versatile sample processing workflow for metagenomic pathogen detection. Sci Rep. 2018;8:13108. PubMed https://doi.org/10.1038/s41598-018-31496-1

2. Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes de novo sssembler. Curr Protoc Bioinformatics. 2020;70:e102. PubMed https://doi.org/10.1002/cpbi.102

3. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics. 2004;5:113. PubMed https://doi.org/10.1186/1471-2105-5-113

4. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–4. PubMed https://doi.org/10.1093/molbev/msaa015

5. Chernomor O, von Haeseler A, Minh BQ. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. 2016;65:997–1008. PubMed https://doi.org/10.1093/sysbio/syw037

6. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017;14:587–9. PubMed https://doi.org/10.1038/nmeth.4285

7. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 2018;35:518–22. PubMed https://doi.org/10.1093/molbev/msx281

and 72°C for 30 sec on a Bio-Rad CFX96 qPCR cycler (Bio-Rad, Germany).

Appendix Table 1. Protein coding genes used for phylogenetic analysis of novel poxvirus from grey seal, Germany*

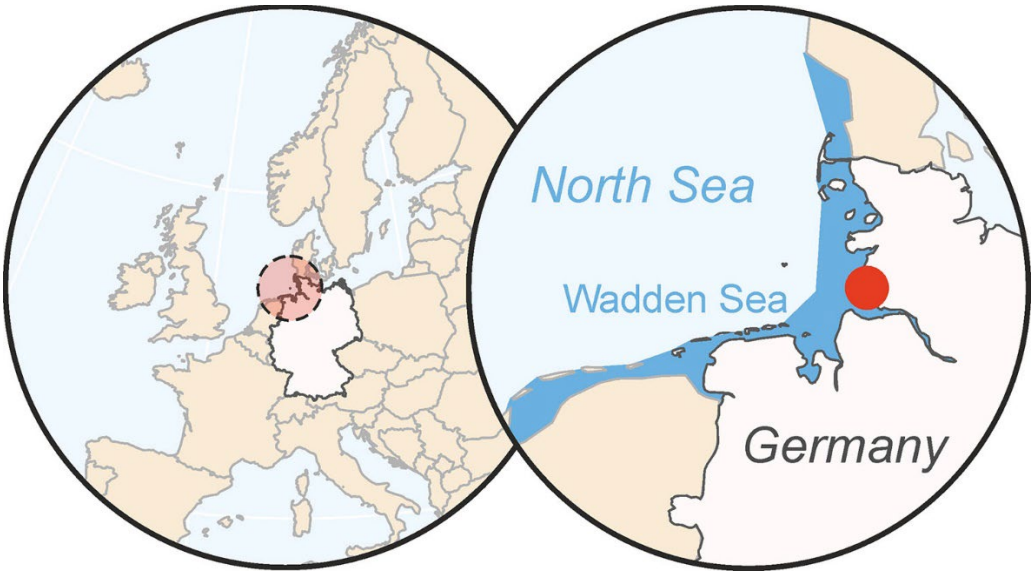
Gene	Gene product	Alignment length, aa
J6R	DNA-dependent RNA polymerase subunit RPO147	1,298
A24R	DNA-dependent RNA polymerase subunit RPO132	1,197
D5R	NTPase	819
E8R	Membrane protein E8	805
A3L	Virion major core protein P4b	789
A18R	DNA helicase	714
D6R	Early transcription factor small subunit VETF-s	656
E6R	IMV membrane protein E6	577
D13L	Trimeric virion coat protein	575
A32L	ATPase	367
D12L	mRNA capping enzyme small subunit	314
G8R	Late transcription factor VLTF-1	268
D4R	Uracil-DNA glycosylase	268
L1R	IMV transmembrane protein	258
A2L	Late transcription factor VLTF-3	233
Total		9,138

*Gene nomenclature is analog to that of Vaccinia virus. Novel poxvirus tentatively named Wadden Sea poxvirus.
aa, amino acid

Appendix Table 2. Sequences of oligonucleotides and hybridization probes used for specific real-time quantitative PCR detection of novel poxvirus from grey seal, Germany*

Oligo ID	Sequence, 5′→3′	Genome position†	Gene
WSPV_Hyb1	6Fam- AGAAACAAATTGTGTGGATCTGGGTTCACA -BHQ1	29901–29930	DNA polymerase
WSPV_F1	GAATTGTTCCCTCTGTTCGTCT	29861–29882	DNA polymerase
WSPV_R1	AAATACCCACCTCCTCAATACA	29963–29942	DNA polymerase
WSPV_Hyb2	6Fam- CGTGCTATATTGGGATGTGCTCAGGCTAAA -BHQ1	103403–103432	RNA polymerase
WSPV_F2	TGTTGCATCATCATTAGTTGGA	103360–103381	RNA polymerase
WSPV_R2	TCAGAACTAAGACACGATATTGCT	103458–103435	RNA polymerase

*Novel poxvirus tentatively named Wadden Sea poxvirus (WSPV).
†Position based on WSPV reference genome, International Nucleotide Sequence Database Collaboration (https://www.insdc.org) accession no. OP810554.



Appendix Figure 1. Map of area where a gray seal (*Halichoerus grypus*) with novel poxvirus was found and nursed, Germany. Map on left shows Germany (white); map on right shows enlarged area of the red circle in the left map and detail of the Waddell Sea area of the North Sea where the seal was found. Red dot indicates location of the rehabilitation facility in northwestern Germany where the gray seal was nursed.

Appendix Figure 2. Verrucous nodules on the right hind flipper of a gray seal (*Halichoerus grypus*) with novel poxvirus, North Sea, Germany. Histopathology of nodules showed severe papillary epithelial hyperplasia with infiltration of neutrophils, ballooning degeneration of epithelial cells, and large eosinophilic intracytoplasmic inclusion bodies (Figure 1).